Effect of Sodium Lauryl Sulfate-Induced Skin Irritation on In Vivo Percutaneous Penetration of Four Drugs

Klaus-P. Wilhelm, Christian Surber, and Howard I. Maibach

Departments of Dermatology, School of Medicine, University of California San Francisco (KPW, SC, HIM), San Francisco, California; and Medizinische Universität zu Lübeck (KPW), Lübeck, Federal Republic of Germany

The influence of sodium lauryl sulfate-induced irritant contact dermatitis on in vivo percutaneous penetration was investigated for four ^{14}C-labeled compounds with diverse physicochemical properties: hydrocortisone (HC), indomethacin (IM), ibuprofen (IB), and acitretin (AC). Hairless guinea pigs were pretreated for 24 h with either 0.5% sodium lauryl sulfate (SLS) to induce irritant contact dermatitis or with water (controls). Twenty-four hours after pretreatment, 450 μl saturated solutions of HC, IM, IB, or AC in isopropylmyristate were applied to the pretreated skin for 24 h. Systemic absorption was determined by urinary and fecal excretion of compounds. Drug concentrations in stratum corneum (obtained by tape cellophane stripping after decontamination of the application site) and in epidermis/dermis (punch biopsy) were also investigated.

Systemic absorption of topically applied drugs (as evaluated by urinary and fecal excretion) in SLS-irritated skin was significantly increased for HC (factor 2.6) followed by IB (1.9 times) and IM (1.6 times) but not increased for AC. However, drug concentrations in the viable epidermis and dermis were 70% lower in SLS-irritated than normal skin for HC, but not different for IB, IM, and AC.

Thus, the influence of the state of the skin (irritant dermatitis versus healthy) on percutaneous penetration was different for diverse drugs. The general assumption that percutaneous penetration and drug tissue concentrations were higher in diseased versus healthy skin was not found to be true in our irritated-skin model. *J Invest Dermatol 97:927-932, 1991*

Sodium lauryl sulfate (SLS) reproducibly induces irritant dermatitis reactions with increased blood flow, TEWL, and mitotic activity [1-4] and has frequently been used as a model substance to study irritant dermatitis [2-6]. We reported increased in vitro penetration for hydrocortisone, indomethacin, ibuprofen, and acitretin in SLS-irritated skin [7]. Flux rates were increased for these compounds to variable extents. Enhancement was higher for hydrophilic compounds than for lipophilic. For all compounds, skin concentrations were increased to a lesser degree than flux through skin. However, in vitro the influence of increased blood flow in irritant dermatitis skin is neglected. Against this background, we investigated percutaneous absorption and skin drug concentration in vivo after topical application of compounds with different physicochemical properties in irritant dermatitis and healthy skin.

Manuscript received August 28, 1990; accepted for publication June 25, 1991.

This work was presented in part at the International Symposium on Controlled Release of Bioactive Materials, 1989, Chicago, IL.

Dr. Surber's present affiliation: Department of Dermatology, Kantonsspital, Basel, Switzerland.

A postdoctoral fellowship for Dr. Wilhelm from the Deutsche Forschungsgemeinschaft (DFG, Federal Republic of Germany) is gratefully acknowledged.

Reprint requests to: Dr. H.I. Maibach, Department of Dermatology, School of Medicine, University of California San Francisco, Box 0989, Surge 110, San Francisco, CA 94143-0989.

Abbreviations:

AC: acitretin
HC: hydrocortisone
IB: ibuprofen
IM: indomethacin
IPM: isoproylmyristate
PBS: phosphate-buffered saline, pH 7.2
Q: cumulative amount compound excreted with urine and feces
D_{skin}: relative skin concentration per 1 cm^2 surface
SC: stratum corneum
SLS: sodium lauryl sulfate
TEWL: transepidermal water loss

MATERIALS AND METHODS

Chemicals Sodium lauryl sulfate was obtained from J.T. Baker Chemicals Co. (Phillipsburg, NJ), hydrocortisone (HC), indomethacin (IM), ibuprofen (IB), and isopropylmyristate (IPM) from Sigma Inc. (St. Louis, MO), [4-^{14}C]-ibuprofen from Boots Co. (Nottingham, UK), [2-^{14}C]-indomethacin and [4-^{14}C]-hydrocortisone from NEN Products (Boston, MA). Acitretin (AC) and [9-^{14}C]-acitretin were a gift from Hoffmann-La Roche (Nutley, NJ). Specific activities were 4.12 mCi/mmol (IB), 56 mCi/mmol (AC), 39.9 mCi/mmol (IM), and 55.0 mCi/mmol (HC). All radiochemicals had a purity greater than 95% (confirmed by thin-layer chromatography with an RTLC scanner [Radiometric Instruments and Chemical Co., Tampa, FL]). Phosphate-buffered saline (PBS) was from the cell culture facility (University of California San Francisco, CA). Skin solubilizer, Soluene-350, was purchased from Packard Instruments Co. Inc. (Downers Grove, IL) and scintillation cocktail, Ready Value, from Beckmann Instruments (Fullerton, CA). To yield maximum thermodynamic activity for each compound, penetration studies were performed with saturated drug solutions (Table I). Drug formulations were prepared as described earlier [7].

0022-202X/91/$03.50

Table I. Investigated Compounds[a]

	HC	IM	IB	AC
Molecular weight	363	358	206	326
Melting point (C°)	218	76	159	226
log [P][b]	1.6	3.1	3.5	6.0
IPM solubility[c]	0.35	2.0	140.0	0.33
mg appl	0.15	0.9	63.0	0.15
μmol appl	0.41	2.51	305.4	0.46

[a] Physicochemical data of compounds used in penetration experiments.
[b] log [P]: octanol water partition coefficient adopted from [16] or experimentally determined (AC).
[c] g/l.

Animals Female hairless guinea pigs (430–550 g) were purchased from Charles River Inc. (Wilmington, MA). Animals were housed at constant ambient conditions (20 ± 2°C; 45–65% rh) with a controlled diurnal cycle and had free access to food and water throughout the experiment. Starting 24 h before exposure until 5 d after exposure, animals were housed in single metabolism cages for separate daily urine and feces collection. Device removal and skin sampling (stratum corneum collection and punch biopsy) were performed under general anesthesia with 10 mg/kg ketamine (Ketaset; Bristol Laboratories, Syracuse, NY) and 2 mg/kg xylazine (Gemini, Rugby Laboratories, Inc., Rockeville Center, NY). This study was approved by the University of California San Francisco Committee on Animal Research.

Pretreatment Previous studies demonstrated that 0.5% SLS induced uniform, moderate to intense erythema in hairless guinea pigs [7]. One half milliliter of 0.5% SLS in aqueous solution was applied to the upper back of the animals with an occlusive plastic chamber (2.5 cm^2; Hilltop, Cincinnati, OH) for 24 h. Distilled water served as control pretreatment.

In Vivo Percutaneous Penetration Four hundred fifty microliters formulation (2 μCi/ml) was applied to the pretreated skin of the guinea pigs with an occlusive polypropylene chamber (2.5 cm^2) for 24 h. The application devices were secured against leakage with

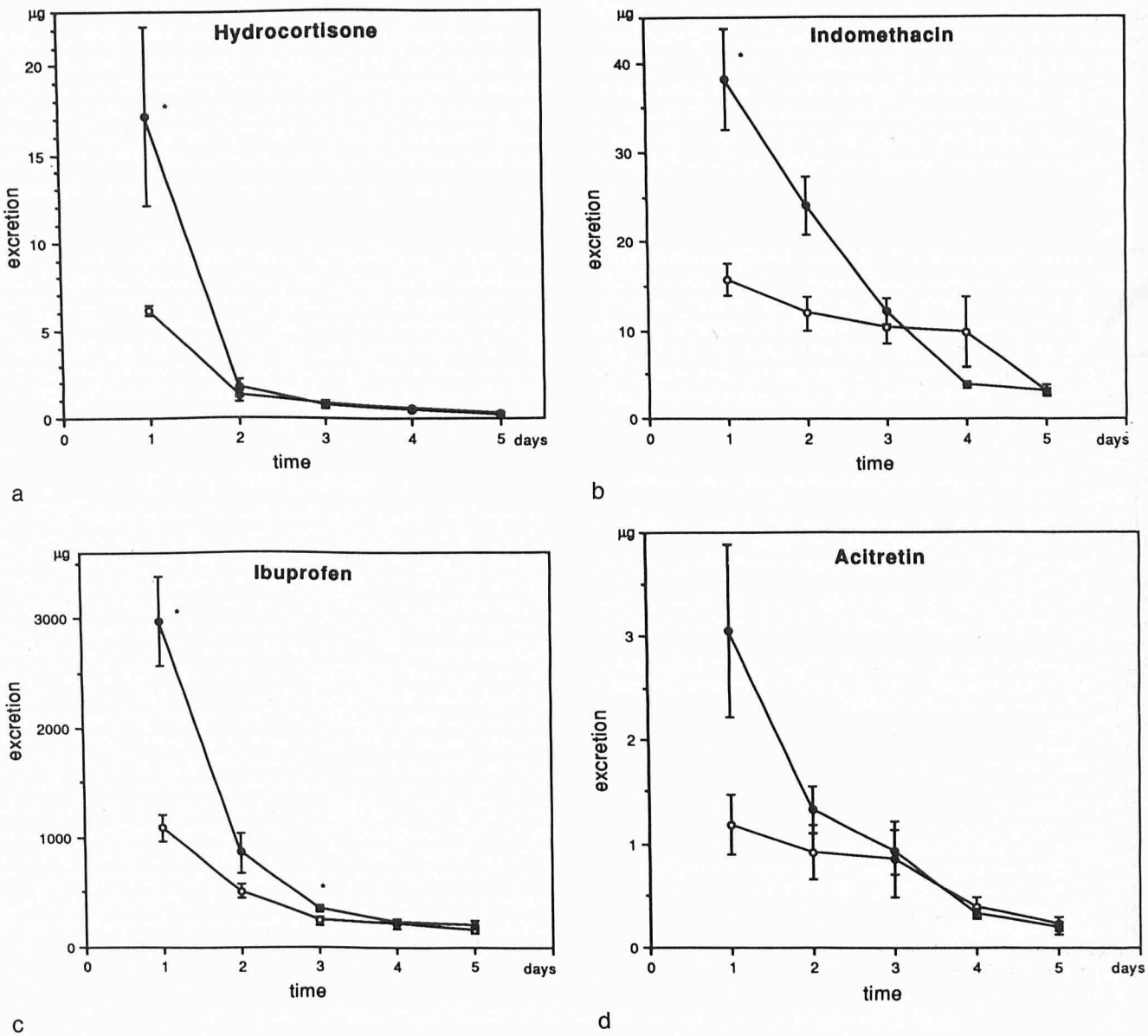

Figure 1. Illustrated are the excretion profiles (urine and feces) over a period of 5 d. Excretion decreases exponentially with time. Significantly (* $p \leq 0.05$) higher values in SLS-irritated animals *(closed circles)* than in controls *(open circles)* were seen for all drugs with the exception of acitretin (mean ± 1 SEM, n = 5–6).

cyanoacrylate glue (Krazy Glue, Krazy Glue, Inc., Itasca, IL) and fastened with non-occlusive paper tape (Scanpore, Norgeplaster, Oslo, Norway).

Sample Handling and Analysis After 24 h, the application device was removed. The application site underwent several washings by a standardized procedure [8] to remove remaining compound. Next, each site received a series of tape strippings. Cellophane tape (Scotch 600, 3M, St. Paul, MN) was pressed to the site. The tape was then withdrawn, leaving stratum corneum on the adhesive. This procedure was repeated 10 times at the site of application. A 6-mm punch biopsy was taken from the center of the application site. The wound was closed with one suture. The entire skin specimen consisting of epidermis and dermis was weighed, and then solubilized in 1 ml skin solubilizer. Urine and fecal samples were collected daily from each animal for 5 d. Samples were collected prior to the application of the radioactive compound to act as a blank for background radiation. Feces were homogenized with water and 0.5 ml aliquots thereof were burned with an oxidizer (X-500; Harvey Instrument Corp., Hilldale, NJ). Radiolabeled $^{14}CO_2$ was trapped in scintillation counter fluid (Carbon 14 Cocktail, C.R.J. Harvey Instrument Corp., Hillsdale, NJ). All samples were quantitated radiometrically with a scintillation counter (TRI-CARB 4640; Pack Instrument Co., Inc., Downers Grove, IL). Prior to counting, all samples were stored at 5°C for 4–7 d after adding scintillation cocktail to allow chemiluminescence to subside.

Calculation and Statistics Relative skin drug concentrations (D_{skin}) were calculated as $D_{skin} = (C_{skin} \times 100)/Q$ and enhancement factors (Ef) as

$$Ef_Q = \frac{Q\ (\text{SLS-group})}{Q\ (\text{control-group})} \text{ or } Ef_{skin} = \frac{C_{skin}\ (\text{SLS-group})}{C_{skin}\ (\text{control-group})},$$

where C_{skin} is drug concentration in epidermis and dermis or in stratum corneum, respectively, and Q is the cumulative amount of drug excreted in urine and feces within 5 d. Statistical comparisons were made with two-tailed Student t test for unpaired data [9] using a statistical package (Primer, version 1.0, McGraw-Hill, Inc., San Francisco, CA) on a personal computer (Macintosh SE 30, Apple Computer, Inc., Cupertino, CA). $p \leq 0.05$ was considered statistically significant.

RESULTS

The excretion profiles of topically applied drugs are illustrated in Fig 1. Percutaneous absorption as evaluated by urinary and fecal excretion was significantly increased in skin pretreated with SLS for hydrocortisone, indomethacin, and ibuprofen (Table II, Fig 2). No significant difference in percutaneous absorption between SLS-irritated skin and controls was noted for acitretin.

Drug levels in SC obtained by tape-stripping the skin are illustrated in Fig 3. The ibuprofen concentration in SC was almost 50 percent lower in SLS-treated animals than in controls. However, no significant differences were seen for the other compounds (Table III)

Drug concentrations in punch biopsy specimens (epidermis and dermis) were not significantly different between irritated and non-irritated skin for indomethacin, ibuprofen, or acitretin but were significantly decreased by 70% for hydrocortisone (Table IV). For topical therapy, high skin drug concentrations with low concomitant systemic absorption should be desirable.

Table II. Percutaneous Penetration[a]

Drug	Control	SLS	Ef_{skin}[b]
HC	9.2 ± 1.28	24.3 ± 10.92[c]	2.6
IM	51.2 ± 19.94	81.1 ± 19.92[c]	1.6
IB	2458.9 ± 446.1	4790.3 ± 1201.9[c]	1.9
AC	4.3 ± 1.80	5.8 ± 2.09[d]	1.4

[a] Shown are amounts of compound excreted (μg) with urine and feces over a 5-d collection period after 24 h application of radiolabeled drugs (means ± SD; n = 5–6). Animals had been pretreated for 24 h with 0.5% SLS or water (controls) respectively.
[b] EF_Q, enhancement ratio.
[c] Significantly different from controls ($p \leq 0.05$, two-tailed t test for unpaired data).
[d] Not significantly different from controls ($p > 0.05$).

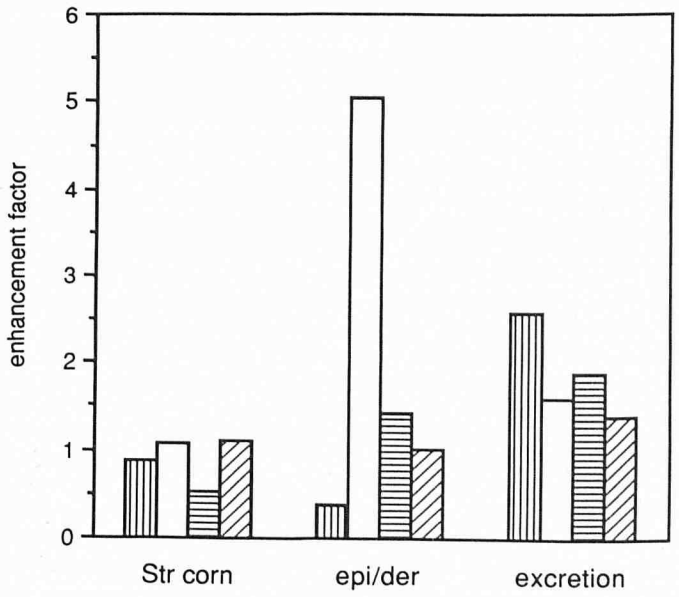

Figure 2. Illustrated are enhancement factors of drug amounts in stratum corneum, epidermis, and dermis and cumulative amount of compound excreted via urine and feces (as a measure for systemic absorption). Enhancement factors were calculated as described in the *Materials and Methods* section. hydrocortisone *(perpendicular shade)*, indomethacin *(open bars)*, ibuprofen *(horizontal shade)*, acitretin *(oblique shade)*. Although the excretion was increased by SLS treatment in the range from 1.4 (AC) to 2.6 (HC), drug levels in stratum corneum were not increased. Drug concentration in epidermis and dermis were less increased than the systemic absorption with the exception of indomethacin.

Comparing relative skin drug concentrations in percent of absorbed dose (D_{skin}), the highest D_{skin} with 4% was seen for hydrocortisone in controls (Fig 4). SLS-irritation, however, significantly decreased this figure to 0.6%. Indomethacin showed the lowest D_{skin} in controls (0.8%) but, in contrast to hydrocortisone, this parameter was significantly higher in SLS-irritated than in non-irritated animals. The amount of drug in the stratum corneum in percent of the absorbed dose was calculated as a measure of a drug's substantivity (Fig 4). The highest substantivity was found for hydrocortisone in healthy skin (22.3%). Substantivity was lower in irritated versus healthy skin for hydrocortisone and ibuprofen but unchanged for indomethacin and acitretin. Mass balance for all experiments was 85% or higher (Table V).

DISCUSSION

We reported earlier the influence of SLS-induced skin irritation on in vitro percutaneous penetration of four drugs [7]. In the present investigation the influence of SLS-induced irritant dermatitis on in vivo percutaneous penetration was studied in vivo for the same drugs: hydrocortisone, indomethacin, ibuprofen, and acitretin. This pretreatment was chosen because SLS is a widely used model substance to study irritant dermatitis [2–6]. In vivo percutaneous absorption in controls differed by less than a factor of 2 (with the exception of acitretin) from in vitro data reported earlier [7]. SLS-irritation increased in vivo percutaneous absorption from 260% (HC) to 140% (AC). These enhancement values are much lower than the corresponding in vitro ratios (range from 600% for hydrocortisone to 400% for acitretin). The opposite might have been expected because increased blood flow is only present in vivo. However, greater effects in vitro than in vivo are also seen when using

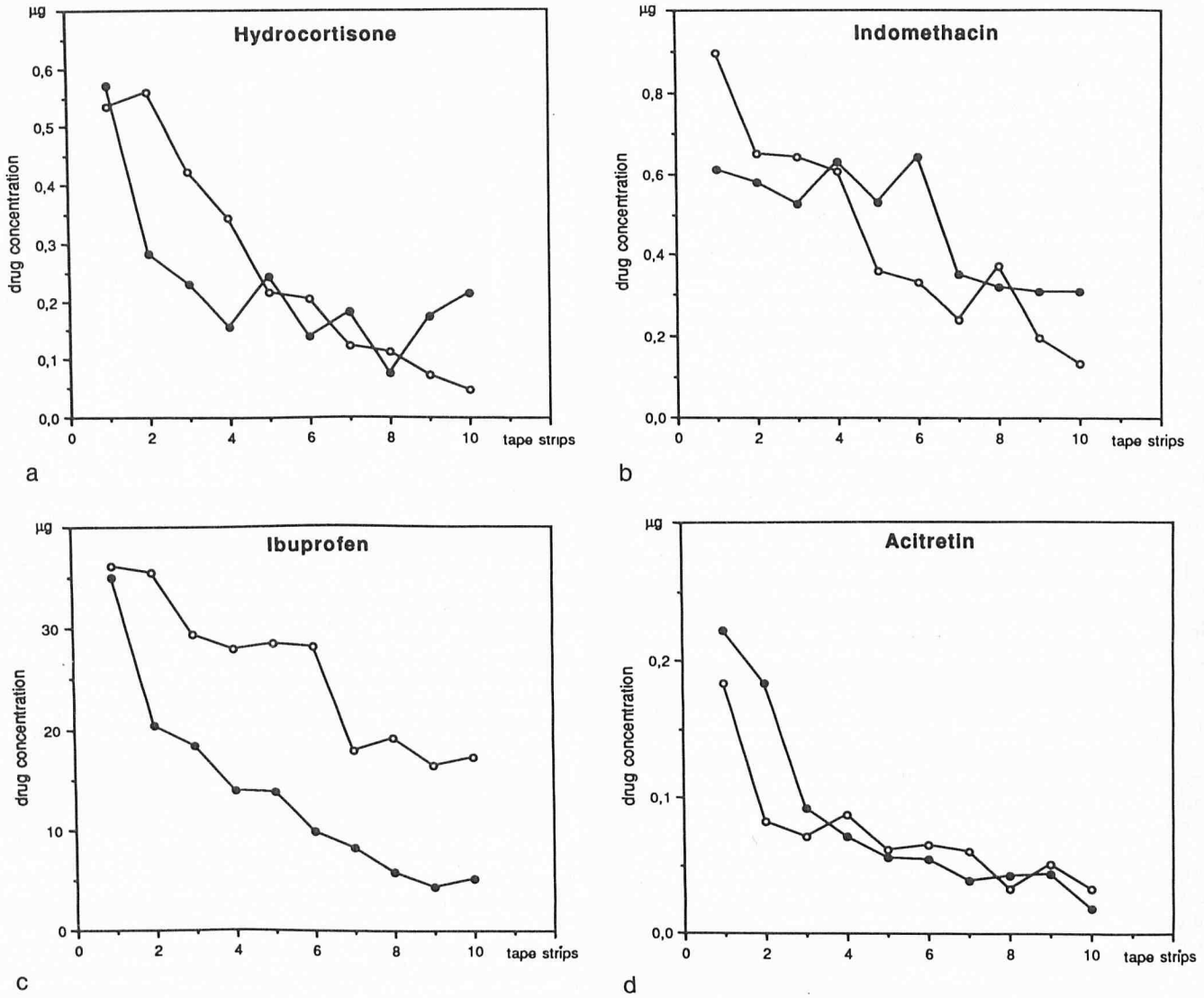

Figure 3. Shown are mean drug concentrations in stratum corneum samples obtained by consecutive tape stripping of the application site (2.5 cm²) after 24 h application of radiolabeled drugs and a washing procedure to remove remaining material from the skin. Drug amount decreases with consecutive stripping for all experiments. Ibuprofen levels in stratum corneum were significantly decreased in SLS-pretreated animals *(closed circles)* as compared with controls *(open circles)*. No significant changes were seen for the other three compounds. For clarity, error bars are not included.

penetration enhancers that intentionally perturb skin barrier properties (R.H. Guy, personal communication). The reason for this phenomenon is still unknown. Another explanation for the smaller enhancement in vivo might be partially related to the healing process in vivo.

More important than differences between in vitro and in vivo experimentation is the mechanistic interpretation of the SLS-induced increase in percutaneous penetration. The most simple way of modeling the process of percutaneous penetration is to assume that Fick's first law of diffusion is applicable although the SC is not an

Table III. SC Drug Concentration[a]

Drug	Control	SLS	Ef_{skin}
HC	2.64 ± 0.98	2.27 ± 1.57[b]	0.86
IM	4.44 ± 2.75	4.81 ± 3.05[b]	1.08
IB	256.14 ± 81.92	134.65 ± 58.97[c]	0.53
AC	0.73 ± 0.58	0.82 ± 0.51[b]	1.12

[a] Drug concentration in stratum corneum (µg drug/2.5 cm²) obtained by tape-stripping after 24 h application of radiolabeled drugs and skin-surface decontamination with soap and water (means ± SD; n = 5–6). Animals had been pretreated for 24 h with 0.5% SLS or water (controls), respectively.
[b] Not significantly different from controls ($p > 0.05$).
[c] Significantly different from controls ($p \leq 0.05$, two-tailed t test for unpaired data).

Table IV. Skin Drug Concentrations[a]

Drug	Control	SLS	Ef_{skin}
HC	1.58 ± 0.94	0.51 ± 0.24[b]	0.32
IM	1.73 ± 0.64	5.61 ± 5.10[c]	3.24
IB	116.86 ± 52.79	163.77 ± 44.66[c]	1.41
AC	0.46 ± 0.14	0.44 ± 0.13[c]	0.96

[a] Shown are drug concentration in puch biopsies (µg drug/g tissue) after 24 h application of radiolabeled drugs and skin-surface decontamination with soap and water and stratum corneum removal by tape stripping (means ± SD; n = 5–6). Animals had been pretreated for 24 h with 0.5% SLS or water (controls), respectively.
[b] Significantly different from controls ($p \leq 0.05$, two-tailed t test for unpaired data).
[c] Not significantly different from controls ($p > 0.05$).

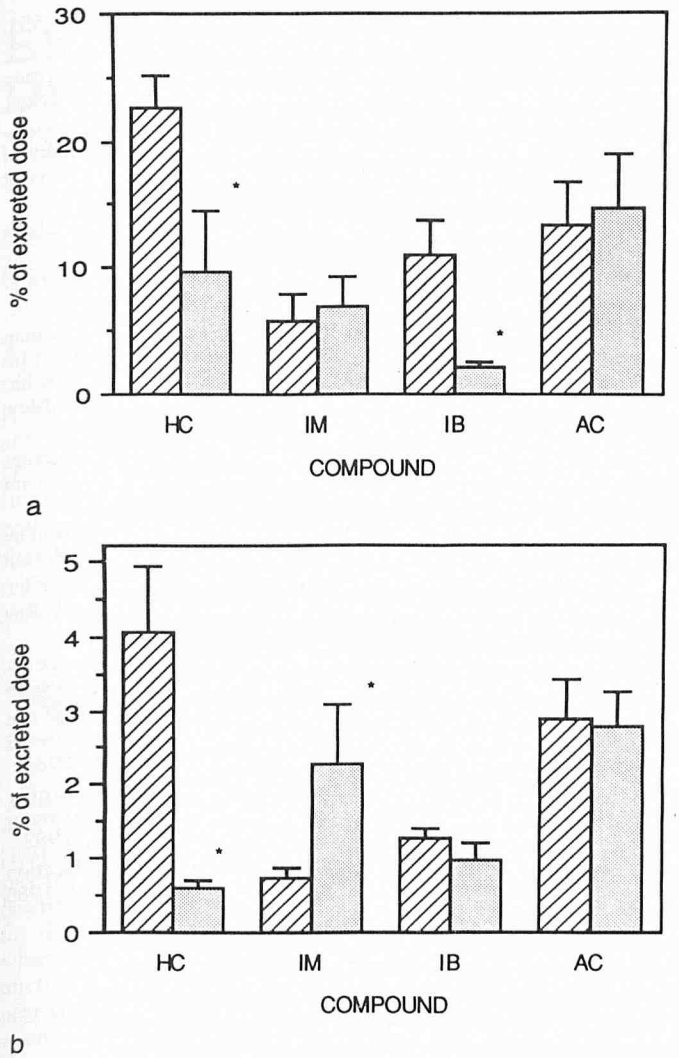

Figure 4. Relative drug concentration in stratum corneum *(a)* (per 2.5 cm^2 surface) and in epidermis and dermis *(b)* (per 2.5 cm^2 surface) in percent of the excreted dose. SLS-pretreatment *(closed bars)* significantly decreased the relative drug dose in stratum corneum for HC and IB as compared with controls *(hatched bars)*. The relative dose in the epidermis and dermis was significantly decreased by SLS-pretreatment whereas a significant increase for IM was seen. *Statistically significant different from controls ($p \leq 0.05$).

inert membrane. The form of the equation often quoted is

$$\frac{dQ}{dt} = \frac{D\,K_p\,c}{h},$$

where dQ/dt is the rate of skin penetration, D is the effective diffusion coefficient of drug in stratum corneum, K_p is the partition coefficient of drug between membrane and solution, c is the concentration gradient of drug, and h is the effective thickness of skin barrier.

c can be considered unchanged by SLS irritation. The remaining three parameters should be discussed separately. It is hypothesized that SLS uncoils and extends α-keratin structure resulting in spatial expansion and increased SC surface and SC thickness [10–13]. Increase of h should result in decreased penetration. However, insertion of SLS into the lipid structure may reduce the ability of the lipids to pack together, resulting in a fluidization of intercellular lipids [11,13]. These mechanisms would increase diffusivity (D). Because the diffusional resistance of SC is greater to polar substances than to nonpolar materials [14–16] a disruption of this barrier should enhance penetration of hydrophilic compounds to a greater extent than the penetration of lipophilic compounds. The present in vivo and earlier in vitro [7] data support this assumption. Drug concentration within SC was not increased for three of the four compounds. Ibuprofen concentration in SC was significantly decreased by SLS pretreatment. Thus increased drug partitioning into SC was not responsible for the increased percutaneous absorption in SLS-induced irritant contact dermatitis.

For topical therapy it is important to deliver effective drug concentrations into the skin, whereas systemic absorption can be regarded as an unintended side effect. Comparing irritated versus healthy skin, it was found that the skin drug concentration in irritated skin was significantly decreased for hydrocortisone but not significantly different for the other three drugs. This unexpected decrease in hydrocortisone skin concentration might partially explain why topical glucocorticosteroids perform poorly in SLS-induced irritant dermatitis [17]. Ideally, for topical therapy D_{skin} should be as high as possible (indicating relatively low levels of systemic absorption). D_{skin} was highest for hydrocortisone in healthy skin. However, in irritant dermatitis D_{skin} decreased significantly for hydrocortisone (meaning a disadvantage for topical therapy). In contrast, D_{skin} was significantly increased for indomethacin, but was unchanged for ibuprofen and acitretin.

Increased drug concentrations in diseased skin as demonstrated for topical dithranol therapy of psoriasis [18] could not be confirmed in our model of irritated skin but for one drug (indomethacin). For other compounds the increase in systemic absorption outweighed the increase in skin concentrations. Our results suggest that topical drug therapy in irritant dermatitis deserves special caution and the

Table V. In Vivo Mass Balance[a]

	Formulation[b]	Skin[c]	Urine	Feces	Total
HC control	81.7 ± 5.99	1.37 ± 0.49	2.7 ± 0.80	3.2 ± 1.05	89.0 ± 5.03
HC + SLS	68.2 ± 6.80	1.09 ± 0.78	10.5 ± 5.44	5.0 ± 1.76	84.7 ± 3.81
IM (control)	85.9 ± 3.75	0.33 ± 0.27	1.6 ± 1.32	4.1 ± 1.29	91.8 ± 3.26
IM + SLS	85.4 ± 2.97	0.70 ± 0.59	2.1 ± 0.42	7.0 ± 1.93	95.3 ± 1.52
IB (control)	90.4 ± 2.91	0.35 ± 0.11	1.7 ± 0.73	2.2 ± 0.32	94.7 ± 2.59
IB + SLS	80.2 ± 3.64	0.17 ± 0.07	4.5 ± 1.81	3.1 ± 0.81	88.0 ± 3.60
AC (control)	85.6 ± 4.06	0.43 ± 0.35	0.5 ± 0.23	2.4 ± 1.32	88.9 ± 4.64
AC + SLS	83.6 ± 4.96	0.41 ± 0.26	0.6 ± 0.10	3.3 ± 1.18	87.9 ± 4.86

[a] Summarized is the recovery of applied substance in percent of applied dose (means ± SD, n = 5–6).
[b] Includes drug remaining in the application device, recovered by washing and first tape strip.
[c] Includes stratum corneum obtained by tape-stripping (strip 2–10) and punch biopsy material.

awareness of possibly increased systemic absorption. In addition, SLS, which is considered as a penetration enhancer for transdermal drug delivery systems, may not be an appropriate additive to topical vehicle formulations to increase dermatologic therapy.

In summary, percutaneous penetration parameter for diverse drugs were differently influenced by SLS-induced irritation. Systemic absorption was increased for three of four drugs. Skin drug concentrations, in contrast, were unchanged for all drugs except for hydrocortisone, for which a significant decrease in SLS-irritated skin was observed. Thus, no simple conclusions nor predictions on the penetration characteristics for diverse drugs in diseased skin can be made. Indeed, further studies for a broader range of drugs for different skin diseases seem justified.

The authors thank Philip Ledger for his help in determining the radiopurity.

REFERENCES

1. Wilhelm KP, Saunders JC, Maibach HI: Increased stratum corneum turnover induced by subclinical irritant dermatitis. Br J Dermatol 122:793–798, 1990
2. Wilhelm KP, Surber C, Maibach HI: Quantification of sodium lauryl sulfate irritant dermatitis in man: comparison of four techniques: skin color reflectance, transepidermal water loss, laser Doppler flow measurement and visual scores. Arch Dermatol Res 281:293–295, 1989
3. Moon KC, Maibach HI: Percutaneous penetration in diseased skin: relationship to exogenous dermatoses. In: Menné T, Maibach HI (eds.). Exogenous Dermatoses: Environmental Dermatitis. CRC Press, Boca Raton, FL, pp 217–226, 1991
4. Van der Valk PJM, Nater JP, Bleumink E: Skin irritancy of surfactants as assessed by water vapor loss measurements. J Invest Dermatol 82:291–293, 1984
5. Bettley FR: The irritant effect of detergents. Trans St Johns Hosp Dermatol Soc 58:65–74, 1972
6. Rietschel RL: Irritant Contact Dermatitis. Dermatol Clin 2:545–551, 1984
7. Wilhelm KP, Surber C, Maibach HI: Percutaneous absorption through irritant dermatitis skin: in vitro studies with four drugs. J Invest Dermatol 96:963–967, 1991
8. Bucks DAW, McMaster JR, Maibach HI, Guy RH: Bioavailability of topically administered steroids a 'mass balance' technique. J Invest Dermatol 90:29–33, 1988
9. Zar JH: Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ, pp 101–120, 1974
10. Scheuplein R, Ross L: Effects of surfactants and solvents on the permeability of epidermis. J Soc Cosmet Chem 21:853–873, 1970
11. Goodman M, Barry BW: Action of penetration enhancers on human stratum corneum as assessed by differential scanning calorimetry. In: Percutaneous Absorption. Bronaugh RL, Maibach HI (eds.). Mechanisms-Methodology-Drug Delivery, 2nd ed. Marcel Dekker, New York and Basel, pp 567–595, 1989
12. Rhein LD, Robbins CR, Ferne K, Cantore R: Surfactant structure effects on swelling of isolated human stratum corneum. J Soc Cosm Chem 37:125–139, 1986
13. Imokawa G, Sumura K, Katsumi M: Study on skin roughness caused by surfactants II. Correlation between protein denaturation and skin roughness. J Am Oil Chem Soc 52:484–489, 1975
14. Scheuplein RJ, Blank IH: Permeability of the skin. Physiol Rev 51:702–747, 1971
15. Guy RH, Hadgraft J: Physicochemical aspects of percutaneous penetration and its enhancement. Pharmaceut Res 5:753–758, 1988
16. Kasting GB, Smith RL, Cooper ER: Effect of lipid solubility and molecular size on percutaneous absorption. In: Shroot B, Schaefer H (eds.). Skin Pharmacokinetics. Karger, Basel, pp 138–153, 1987
17. Van der Valk PGM, Maibach HI: Do topical corticosteroids modulate skin irritation in human beings? Assessment by transepidermal water loss and visual scoring. J Am Acad Dermatol 21:519–522, 1989
18. Schaefer H, Farber EM, Goldberg L, Schalla W: Limited application period for dithranol in psoriasis. Br J Dermatol 102:571–573, 1980